

Appl. No.: 10/675,011
Filed: September 30, 2003
Amdt. Dated November 2, 2007

Amendments to the Specification:

Please replace the "CROSS-REFERENCE TO RELATED APPLICATIONS" paragraph at page 1 with the following amended paragraph:

This application is a continuation-in-part of U.S. Application Serial No. 09/915,873, filed July 26, 2001, now U.S. Patent No. 6,815,184; which claims the benefit of U.S. Provisional Application Serial No. 60/221,705, filed July 31, 2000, and U.S. Provisional Application Serial No. 60/293,330, filed May 23, 2001; each of which is hereby incorporated in its entirety by reference herein.

On page 13, please replace the paragraph beginning at line 3 with the following amended paragraph:

"*Lemna gibba*-preferred codons" as used herein refers to codons that have a frequency of codon usage in *Lemna gibba* of greater than 17% where the frequency of codon usage in *Lemna gibba* was obtained from the Codon Usage Database (GenBank Release 113,0; at [http://www.kazusa.or.jp/codon/cgi-bin/showcodon.cgi?species=Lemna+gibba+\[gbpln\]](http://www.kazusa.or.jp/codon/cgi-bin/showcodon.cgi?species=Lemna+gibba+[gbpln]) the website at [kazusa.or.jp](http://www.kazusa.or.jp)).

On page 18, please replace the paragraph beginning at line 13 with the following amended paragraph:

The present invention provides for the modification of the expressed nucleotide sequence to enhance its expression in duckweed. One such modification is the synthesis of the nucleotide sequence of interest using duckweed-preferred codons. Methods are available in the art for synthesizing nucleotide sequences with plant-preferred codons. See, e.g., U.S. Patent Nos. 5,380,831 and 5,436,391; Perlak *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 15:3324; Iannacome *et al.* (1997) *Plant Mol. Biol.* 34:485; and Murray *et al.*, (1989) *Nucleic Acids. Res.* 17:477, herein

incorporated by reference. The preferred codons may be determined from the codons of highest frequency in the proteins expressed in duckweed. For example, the frequency of codon usage for *Lemna gibba* is found on the ~~web page: [http://www.kazusa.or.jp/codon/cgi-bin/showcodon.cgi?species=Lemna+gibba+\[gbpln\]](http://www.kazusa.or.jp/codon/cgi-bin/showcodon.cgi?species=Lemna+gibba+[gbpln])~~ website at [kazusa.or.jp](http://www.kazusa.or.jp), and the frequency of codon usage for *Lemna minor* is found on the ~~web page [http://www.kazusa.or.jp/codon/cgi-bin/showcodon.cgi?species=Lemna+minor+\[gbpln\]](http://www.kazusa.or.jp/codon/cgi-bin/showcodon.cgi?species=Lemna+minor+[gbpln])~~ website at [kazusa.or.jp](http://www.kazusa.or.jp) and in Table 1. It is recognized that genes that have been optimized for expression in duckweed and other monocots can be used in the methods of the invention. See, e.g., EP 0 359 472, EP 0 385 962, WO 91/16432; Perlak *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:3324; Iannacome *et al.* (1997) *Plant Mol. Biol.* 34:485; and Murray *et al.* (1989) *Nuc. Acids Res.* 17:477, and the like, herein incorporated by reference. It is further recognized that all or any part of the gene sequence may be optimized or synthetic. In other words, fully optimized or partially optimized sequences may also be used. For example, 40 %, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the codons may be duckweed-preferred codons. In one embodiment, between 90 and 96 % of the codons are duckweed-preferred codons. The coding sequence of the nucleotide sequence of interest may comprise codons used with a frequency of at least 17% in *Lemna gibba*. In one embodiment, the modified nucleotide sequence is the human α -2B-interferon encoding nucleotide sequence shown in SEQ ID NO:2, which contains 93% duckweed preferred codons.

On page 24, please replace the paragraph beginning at line 12 with the following amended paragraph:

The comparison of sequences and determination of percent identity and percent similarity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (1970) *J. Mol. Biol.* 48:444-453 algorithm, which is incorporated into the GAP program in the GCG software package (available at ~~www.accelrys.com~~ the website at accelrys.com), using either a BLOSSUM62 matrix or a PAM250 matrix, and a gap weight of 16,

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14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package, using a BLOSUM62 scoring matrix (see Henikoff *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 89:10915) and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used if the practitioner is uncertain about what parameters should be applied to determine if a molecule is within a sequence identity limitation of the invention) is using a BLOSUM62 scoring matrix with a gap weight of 60 and a length weight of 3).